THROMBOXANE SYNTHETASE INHIBITION AND PULMONARY RESPONSE TO HYPOXIA IN CONSCIOUS ADULT SHEEP

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This study investigated the effects of a thromboxane synthetase inhibitor (OKY-046) and a cyclooxygenase inhibitor (ketoprofen) on hypoxic pulmonary vasoconstriction in conscious adult sheep in order to evaluate the physiological role of thromboxane and other cyclooxygenase products. In addition, we studied the effects of histamine H₁ (chlorpheniramine) and H₂ antagonists (cimetidine) on hypoxic pulmonary vascular tone. Hypoxia caused a 37% rise in pulmonary arterial pressure ($p < 0.05$) and a 36% increase in pulmonary vascular resistance ($p < 0.05$). Pretreatment with intravenous OKY-046 10 mg/kg or ketoprofen 2 mg/kg had no effect on normoxic pulmonary vascular tone and inhibited the increase in plasma TXB₂ concentration during hypoxia without affecting the pulmonary pressor response to hypoxia. Cimetidine produced an increase in hypoxic pulmonary vascular tone when individual members of the group were compared, but there was no statistically significant difference when the group was compared to the control study. Chlorpheniramine had no effect on hypoxic pulmonary tone. These data suggest that hypoxic pulmonary vasoconstriction is not mediated by release of TXA₂, that hypoxic vascular tone is not modulated by cyclooxygenase products, and that the histamine H₂ receptor may play a modulating role in hypoxic pulmonary vasoconstriction in conscious adult sheep.

ALTHOUGH alveolar hypoxia elicits pulmonary vasoconstriction, the mechanism of this response has not been explained.¹ Intrapulmonary prostaglandin (PG) synthesis is influenced by various stimuli, including hypoxia, and prostanooids may act as modulators of pulmonary vasoconstriction. Effects of PG cyclooxygenase inhibition on hypoxic pulmonary vascular tone have been previously reported; however, there

is some conflicting evidence. Said et al² have demonstrated that inhibition of PG biosynthesis by aspirin reduced the pulmonary vasoconstrictor response to hypoxia in anesthetized cats. PG synthesis inhibition by meclofenamate or indomethacin increased the pulmonary pressor responses to hypoxia in anesthetized dogs.³ Pretreatment with indomethacin caused hypoxic pulmonary vasoconstriction in the "nonresponders", which were only a small number of sheep (approximately 8%); the drug did not potentiate the hypoxic pulmonary response in conscious sheep "responders".⁴

In order to assess the role of thromboxane

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(TX) A₂ in hypoxic pulmonary vasoconstriction, we examined the effects of OKY-046 [a selective TX synthetase inhibitor, which can inhibit synthesis of the potent vasoconstrictor substance TXA₂, without affecting biosynthesis of PGI₂⁵] and ketoprofen [a PG cyclooxygenase inhibitor⁶], on acute hypoxic pulmonary vasoconstriction. In addition, we studied another possible explanation, histamine receptors, which may underlie the hypoxic pulmonary vasodilation response. In this study, we used conscious sheep which have adequate acute hypoxic pulmonary vasoconstriction,⁷ in order to eliminate the effect of anesthesia, which alters PG biosynthesis⁸ and the responses to physiologic and pharmacologic stimuli⁹.

MATERIALS AND METHODS

Sheep of either sex, weighing between 25 and 44 kg were used. They were anesthetized with intravenous pentobarbital sodium (20 mg/kg) and intubated with a cuffed endotracheal tube. The animals were ventilated with 1% halothane using an animal respirator (Harvard Apparatus Co., S. Natick, MA, U.S.A.). Silicon tubes (ID; 2 mm, OD; 4 mm) were inserted into the thoracic aorta and the right atrium through the left carotid artery and the right jugular vein, respectively. A left thoracotomy (3rd intercostal space) was performed under aseptic conditions, and the tubes were directly inserted into the pulmonary artery and the left atrium. An electromagnetic flow probe (Narco Co.) was placed around the trunk of the pulmonary artery. An antibiotic was administered daily for five days following surgery. The tubes were thoroughly flushed with normal saline and filled with 2 ml heparin once a day. Approximately one week later, when the effects of the operation seemed to have almost subsided, the following experiments were performed in the conscious sheep. During the experiments the sheep were supported and fixed by a sling in a breeding cage, and their heads immobilized with a collar. After topical anesthesia of the nasal passage with a 1% lidocaine solution, a cuffed nasotracheal tube coated with 2% lidocaine jelly was inserted through a nostril.

Mean pulmonary arterial pressure (Ppa) and mean left atrial pressure (Pla) were continuously measured using pressure transducers (Model MPU-0.5-290-III, Nihon Koden Co., Tokyo, Japan), the level at the left atrium being taken as the zero reference. The cardiac output (CO) was

measured with an electromagnetic flowmeter (Narco RT-500, Narco Co., Houston, TX, U.S.A.). Parameters were recorded on an eight-channel recorder (Model WT-685G, Nihon Koden Co.). Pulmonary vascular resistance (PVR) was calculated as follows; $PVR = (Ppa - Pla) / CO$.

Arterial blood (1 ml) was drawn from the thoracic aorta using a heparinized syringe, and the blood samples analyzed at 37°C for pH, arterial oxygen pressure (PaO₂) and carbon dioxide pressure (PaCO₂) with a pH/blood gas analyzer (Model ABL-2; Radiometer Co., Copenhagen, Denmark). Blood samples (5 ml) for analysis of plasma TXB₂ and 6-keto-PGF_{1α} were simultaneously drawn from the pulmonary artery and the left atrium and transferred into plastic tubes containing 50 μl of heparin sodium (1,000 U/ml; Upjohn Diagnostic Laboratories, Kalamazoo, MI, U.S.A.) and 50 μl of 10⁻⁵ molar indomethacin. These samples were immediately placed on ice, then centrifuged at 2,500g at 4°C for 10 min and frozen at -30°C. The TXB₂ and 6-keto-PGF_{1α} were extracted according to the modified methods of Tada et al.¹⁰ and Jaffe et al.¹¹ respectively, and measured in duplicate by the radioimmunoassay using labeled (5, 6, 8, 9, 11, 12, 14, 15-H³) TXB₂ and 6-keto-(5, 6, 8, 9, 11, 12, 14, 15-H³) PGF_{1α} purchased from New England Nuclear Co. (Boston, MA, U.S.A.).

EXPERIMENTAL PROTOCOLS

Hemodynamic effects of hypoxia: A hypoxic control study was performed in six sheep to ascertain the effects of time alone on the hemodynamic responses to hypoxia. Baseline measurements of all parameters were obtained after the animals had rested for 20 to 30 min, breathing room air through the nasotracheal tube. The sheep were then switched to a hypoxic gas mixture of 10% oxygen in nitrogen. Measurements were continued for 40 min under hypoxic ventilation.

Modification of the hypoxic response by thromboxane synthetase inhibitors: To evaluate the effects of TX synthetase inhibitors on hypoxic pulmonary vascular tone, six animals were pretreated with 10 mg/kg of OKY-046 (Kissei Pharmaceutical Co., Matsumoto, Japan) introduced into the right atrium during room air ventilation. OKY-046 was dissolved in normal saline to a concentration of 20 to 30 mg/ml just prior to use. After 10 min of the pretreatment, continuous hypoxic ventilation was begun and

TABLE I EFFECTS OF OKY-046 AND KETOPROFEN ON HYPOXIC PULMONARY PRESSOR RESPONSE

	Control study			OKY-046			Ketoprofen		
	Baseline	Hypoxia		Baseline	Postdrug	Hypoxia	Baseline	Postdrug	Hypoxia
Ppa (mmHg)	14.5 ± 0.5	19.8 ± 0.6*		14.5 ± 0.7	14.3 ± 0.6	19.9 ± 0.6*	13.1 ± 0.7	13.4 ± 0.8	18.1 ± 1.5*
Pla (mmHg)	4.8 ± 0.3	3.3 ± 0.4*		4.6 ± 0.7	4.5 ± 0.8	3.1 ± 0.5*	3.8 ± 0.3	3.7 ± 0.4	2.8 ± 0.7
CO (L/min)	2.76 ± 0.15	3.55 ± 0.25*		3.30 ± 0.22	3.31 ± 0.25	3.98 ± 0.12*	3.22 ± 0.24	3.32 ± 0.17	4.02 ± 0.28*
PVR (mmHg/L/min)	3.54 ± 0.22	4.80 ± 0.41*		3.02 ± 0.14	2.97 ± 0.13	4.22 ± 0.29*	2.93 ± 0.26	2.88 ± 0.25	3.82 ± 0.26*
PaO ₂ (mmHg)	93.5 ± 4.6	34.2 ± 2.4*		98.3 ± 6.8	98.2 ± 3.0	32.9 ± 3.4*	97.8 ± 1.0	99.2 ± 4.1	36.1 ± 2.6*
PaCO ₂ (mmHg)	30.7 ± 1.2	22.6 ± 0.7*		30.5 ± 2.3	27.3 ± 0.8	22.6 ± 1.2*	28.0 ± 0.9	26.4 ± 0.4	27.1 ± 1.1
pH	7.51 ± 0.01	7.55 ± 0.03*		7.46 ± 0.03	7.49 ± 0.01	7.55 ± 0.02*	7.52 ± 0.02	7.52 ± 0.01	7.56 ± 0.02*

Definition of abbreviations: Ppa = mean pulmonary arterial pressure; Pla = mean left atrial pressure; CO = cardiac output; PVR = pulmonary vascular resistance; PaO₂ = arterial oxygen tension; PaCO₂ = arterial carbon dioxide tension.

Data are means and SEM.

*p < 0.05 from baseline in the control study and from postdrug values in the OKY-046 and ketoprofen groups.

the measurement of all parameters continued for 40 min after the start of the hypoxic condition.

Modification of the hypoxic response by cyclooxygenase inhibitors: To evaluate the effects of cyclooxygenase inhibitors on hypoxic pulmonary vascular tone, six animals were pretreated with 2 mg/kg of ketoprofen (Kissei Pharmaceutical) introduced into the right atrium during room air ventilation. Ketoprofen was first dissolved in 2.5 ml of 1% benzyl alcohol and normal saline added to bring the dose to a total volume of 10 ml. After 30 min of the pretreatment,¹² a continuous hypoxic ventilation was begun and the measurement of all parameters continued for 40 min after the start of the hypoxic condition.

Arterial blood gas and prostanoid analysis were performed in all three groups at the end of each experimental stage.

Furthermore, we studied modifications of the hypoxic response by histamine H₁- or H₂-receptor antagonists after induction of hypoxic vasoconstriction. After the baseline measurements, continuous hypoxic ventilation (10% oxygen) was performed. Twenty minutes after the beginning of the hypoxic condition, six animals were given a 2 mg/kg-bolus of chlorpheniramine malate (Shionogi Pharmaceutical Co., Osaka, Japan), and five animals received a 10 mg/kg-bolus of cimetidine (Fujisawa Pharmaceutical Co., Osaka, Japan). Measurements were continued for an additional 20 min of hypoxia after the administration of each drug.

STATISTICAL ANALYSIS

Data are presented as the means and standard errors of the mean. Comparison between baseline and experimental values in each sheep was performed using the Student t-test for paired data. Comparisons between the different groups were done using the unpaired t-test. A confidence level greater than 99–95% (p < 0.05) was considered statistically significant.

RESULTS

Pulmonary hemodynamic responses to hypoxia and the effects of OKY-046 and ketoprofen on hypoxic pulmonary response are shown in Table I. Hypoxia caused a 37% rise in pulmonary arterial pressure (p < 0.05) and a 36% increase in pulmonary vascular resistance (p < 0.05) in the hypoxic control study. These significant

Fig.1. The effects of histamine receptor antagonists (HRA) given during hypoxia on pulmonary arterial pressure and pulmonary vascular resistance. There was no difference between the groups at each stage of the study.

TABLE II PLASMA CONCENTRATIONS OF TXB_2 (pg/ml) AND 6-KETO-PGF $_{1\alpha}$ (pg/ml) IN HYPOXIC CONTROL STUDY, OKY-046 AND KETOPROFEN PRETREATED SHEEP

	TXB_2		6-Keto-PGF $_{1\alpha}$	
	Pulmonary artery	Left atrium	Pulmonary artery	Left atrium
Baseline				
Control	142 ± 23	138 ± 17	21 ± 8	30 ± 10
OKY-046	173 ± 29	166 ± 26	32 ± 9	24 ± 3
Ketoprofen	88 ± 8	111 ± 18	12 ± 2	11 ± 1
Hypoxia				
Control	231 ± 33*	185 ± 20*	42 ± 6	35 ± 10
OKY-046	119 ± 4#	117 ± 7#	23 ± 8	25 ± 4
Ketoprofen	105 ± 15#	110 ± 22#	11 ± 3#	9 ± 1#

Data are means and SEM.

* $p < 0.05$ from baseline.

$p < 0.05$ between control and OKY-046 or ketoprofen pretreated group.

risks during hypoxic ventilation remained stable for 40 min. Hypoxia significantly lowered PaO_2 to a level of less than 40 mmHg ($p < 0.05$; mean $\text{PaO}_2 = 34.2$ mmHg).

The pulmonary hemodynamics, arterial blood gas pressures and pH obtained after the acute administration of both OKY-046 and ketoprofen during room air ventilation were similar to the baseline values. In the OKY-046 pretreatment group, hypoxia significantly increased Ppa and

PVR by 39% ($p < 0.05$) and 42% ($p < 0.05$), respectively. Similarly in the ketoprofen pretreatment group, hypoxic ventilation produced significant increases in Ppa and PVR by 35% ($p < 0.05$) and 33% ($p < 0.05$), respectively. Mean PaO_2 values during hypoxic conditions were similar with or without these pretreatments. The hypoxia-induced increases in Ppa and PVR were unaffected by OKY-046 or ketoprofen pretreatment.

The effects of histamine H_1 - and H_2 -receptor antagonists on pulmonary vascular tone when given after 20 min of hypoxic ventilation are shown in Fig. 1. Hemodynamic parameters of all three groups were comparable during room air and 20 min of hypoxic ventilation. Cimetidine produced increases in Ppa and PVR when individual members of the group were compared (hypoxia vs hypoxia + HRA; $p < 0.05$), but there was no statistically significant difference when compared with those in the control study or chlorpheniramine groups at each stage of the study.

The changes in plasma concentrations of TXB_2 and 6-keto-PGF $_{1\alpha}$ are shown in Table II. Plasma samples were drawn simultaneously from the pulmonary artery (PA) and the left atrium (LA). Although ketoprofen pretreatment revealed a tendency to reduce plasma TXB_2 of PA in the baseline period, the decrease was not statistically significant. Hypoxic ventilation significantly increased plasma TXB_2 of both PA and LA in the hypoxic control study; however, 6-keto-PGF $_{1\alpha}$ remained nearly constant. In both OKY-046 and ketoprofen pretreatment groups, hypoxia did not cause the increases in plasma TXB_2 concentrations of PA and LA, which were observed in the hypoxic control study.

DISCUSSION

Pulmonary pressor response to hypoxia has been observed in most animal species, although the mechanism is not clear.¹ Hypoxic pulmonary vasoconstriction is not believed to be produced by prostaglandins.³ Previous reports in newborn goats using an isolated perfusion technique¹³ have suggested that prostaglandin synthesis inhibition by indomethacin may augment pulmonary pressor response to hypoxia, indicating removal of the vasodilator effect of the prostaglandin during hypoxia. No significant difference was observed in the hypoxic pulmonary response with or without thromboxane synthesis inhibition in lambs.¹⁴

In these results, hypoxic ventilation produced pulmonary vasoconstriction with an increase in the plasma TXB_2 concentration in the control study. OKY-046 effectively inhibited the increase in plasma TXB_2 concentration during hypoxia; however, the hypoxic pulmonary vascular tone was unaffected by these drugs in conscious adult sheep. These results suggest that TXA_2 cannot play a mediating role in hypoxic

pulmonary vasoconstriction. The observation that the decrease in plasma 6-keto-PGF $_{1\alpha}$ concentration in the ketoprofen pretreatment group when compared to the control study further suggests that the prostacyclin and other endogenous cyclooxygenase products do not have modulating effects of hypoxic pulmonary vascular tone. There is some conflicting evidence within our results. Prostaglandin-like substances have been shown to be present in the efflux of pulmonary perfusate during hypoxia in isolated perfused cat lungs, and a cyclooxygenase inhibitor, aspirin, reduced hypoxic pulmonary vasoconstriction in anesthetized cats.² On the other hand, reports in agreement with our data have recently demonstrated that indomethacin and meclofenamate,¹⁵ and thromboxane synthetase inhibitor¹⁴ have no effect on hypoxic pulmonary vascular tone.

The endogenous vasodilator prostaglandins are believed to be involved in maintaining a normal low pulmonary vascular tone.³ The concept that responses to indomethacin of normoxic pulmonary vascular tone may be related to the effectiveness of cyclooxygenase products inhibition during normoxia could be proposed. The acute intravenous administration of indomethacin (5 mg/kg) produced significant increases in pulmonary vascular resistance during normoxia, although subacute administration of indomethacin produced no effect on normoxic tone.¹⁵ In these results, ketoprofen (2 mg/kg) produced no effect on normoxic pulmonary vascular tone, although showing tendencies to decrease plasma TXB_2 and 6-keto-PGF $_{1\alpha}$ which were not statistically significant. The effects of ketoprofen on the release of immunoreactive prostaglandins from guinea-pig lungs were similar to those of indomethacin and meclofenamate.⁶ Indomethacin produced no effect on normoxic pulmonary vascular resistance in "responders" among the conscious sheep.⁴ Therefore the lack of response to ketoprofen during normoxic vascular tone in our data may be related to the low dose of ketoprofen. Our results suggest that thromboxane A_2 and other vasoconstrictor prostaglandins cannot act as mediators of hypoxic pulmonary vasoconstriction and vasoactive cyclooxygenase products do not play a role in the modulation of normoxic and hypoxic pulmonary vascular tone in conscious adult sheep.

Of the various chemical mediators released from mast cells, histamine has been proposed as a candidate for the mediator of hypoxic pulmo-

nary vasoconstriction. Some reports suggest that the use of antihistamines or depletion of histamine inhibits the pressor response to hypoxia,^{16,17} although there is conflicting evidence.^{18,19} In this study, pulmonary hemodynamic parameters of the control, chlorpheniramine and cimetidine groups were comparable during room air ventilation and 20 min of hypoxia. Cimetidine produced increases in pulmonary arterial pressure and pulmonary vascular resistance when compared within the group (hypoxia vs hypoxia + HRA; $p < 0.05$, Fig. 1); however, there was no statistically significant difference between the three groups. These data suggest that although histamine may modulate hypoxic pulmonary vasoconstriction in conscious adult sheep, it is not the mediator.

In summary, alveolar hypoxia produced pulmonary vasoconstriction with increase in plasma TXB_2 concentration. Thromboxane synthetase inhibitor and cyclooxygenase inhibitor had no effect on normoxic pulmonary vascular tone and inhibited the increase in plasma TXB_2 concentration during hypoxia without affecting the hypoxic pulmonary pressor response. Histamine H_2 receptor blocker produced increases in hypoxic pulmonary vascular tone when compared within the group, although there was no statistically significant difference between the control group. Histamine H_1 -receptor blocker produced a tendency to increase pulmonary vascular resistance though this increase was not statistically significant. These observations suggest that thromboxane A_2 and other cyclooxygenase products do not play mediating and modulating roles, and that histamine H_2 -receptor may play a modulating role in hypoxic pulmonary vasoconstriction in conscious adult sheep.

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